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Clinicopathologic and Survival Correlates of Embryonal Rhabdomyosarcoma Driven by *RAS/RAF* Mutations

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Abstract

Embryonal rhabdomyosarcoma (ERMS) is the most common subtype of rhabdomyosarcoma (RMS). Amongst RMS subtypes, ERMS is associated with a favorable outcome with an overall survival of 70% at 5 years for localized disease. The molecular profile of ERMS is heterogeneous, including mostly point mutations in various genes. Therapeutic strategies have remained relatively consistent irrespective of the molecular abnormalities. In this study, we focus on a homogeneous *RAS/RAF* mutated ERMS subset and correlate with clinicopathologic findings. Twenty-six cases (16 males and 10 females) were identified from screening 98 ERMS, either by targeted DNA sequencing (MSK-IMPACT) or by Sanger sequencing. Fourteen (54%) cases had *NRAS* mutations, 6 (23%) had *KRAS* mutations, 5 (19%) had *HRAS* mutations and 1 case (4%) had *BRAF* mutation. Median age at diagnosis was 8 years (range 1-70) with two-thirds occurring in the children. Tumor sites varied with H&N and GU sites accounting for 62% of cases. *RAS* isoform hot spot mutations predominated: *NRAS p.Q61K* (57%), *KRAS p.G12D* (67%) and *HRAS* (codons 12, 14 and 61). Additional genetic abnormalities were identified in 85% of the *RAS*-mutated cases. At last follow-up, 29% patients died of disease and 23% were alive with disease. The 3-year and 5-year survival rates were 75% and 61% respectively. In conclusion, *RAS* mutations occur in 27% of ERMS, with *NRAS* mutations encompassing half of the cases. Overall *RAS*-mutant RMS do not correlate with age or site, but most tumors show an undifferentiated and spindle cell morphology.

Keywords

NRAS; KRAS; HRAS; Embryonal Rhabdomyosarcoma

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INTRODUCTION

Rhabdomyosarcomas (RMS) are the most common soft tissue sarcomas in children, accounting for 5-8% of all pediatric malignancies, with a small subset occurring in adults as well. There are now 4 subtypes of RMS recognized in the latest WHO classification of soft tissue tumors,[1] mainly defined based on their genetic profiles, including fusion-negative ERMS, *PAX3/7-FOXO1* fusion-positive alveolar RMS (ARMS), spindle and sclerosing RMS with either *MYOD1* mutations or various gene fusions, and pleomorphic RMS. While ARMS and *MYOD1*-mutant spindle and sclerosing RMS follow a highly aggressive clinical course, the prognosis of ERMS has significantly improved in recent years with an overall survival of 70% at 5 years for patients presenting with localized disease. Based on large genomic studies,[2-4] the molecular landscape of ERMS has emerged as driven by a wide range of causative mutations, with most frequent single nucleotide point mutations detected within one of the Ras genes (*NRAS* > *KRAS* > *HRAS*), a receptor tyrosine kinase (*FGFR4* ≫ *ERBB2*), or the catalytic component (*PIK3CA*) of the phosphoinositide-3 kinase (PI3K) complex.[3] As the most common molecular subset of ERMS appears to be driven by an oncogenic mutation in one of the Ras isoforms, the aim of this study is to investigate the potential clinical and pathologic correlates associated with this genotype, as well as its pattern of additional co-existing mutations and impact on survival and response to standard therapy protocols.

MATERIALS AND METHODS

Patient Selection

Archival and consultation material from adult and pediatric patients with a diagnosis of ERMS where additional testing identified *RAS / RAF* mutations was retrieved from the pathology files at Memorial Sloan Kettering Cancer Center. Overall, 93 cases of ERMS were identified in which next-generation targeted DNA sequencing (MSK-IMPACT) was performed. In order to increase the study group, archival material from an additional cohort of 5 ERMS, which preceded the clinical MSK-IMPACT testing (2010-2014), was studied by targeted DNA PCR and Sanger sequencing for hot spot mutations in the 3 RAS genes. Overall, 26 ERMS were identified harboring RAS mutations, with 22 cases being detected by MSK-IMPACT and 4 by Sanger sequencing. The pathologic features of these cases were re-reviewed and analyzed for cell morphology, presence of rhabdomyoblasts, pleomorphism or anaplasia, mitotic activity, and necrosis. The diagnosis of RMS was confirmed by positivity for desmin (Ventana, DE-R-11, RTU) and myogenin (Ventana, F5D, RTU) in all cases. Clinical Risk Group was assessed based on current guidelines[5, 6] and follow-up data was collected by review of clinical records. The study was approved by the Institutional Review Board at MSKCC (IRB# 02-060).

MSK-IMPACT Assay

Details of the MSK-IMPACT assay have been previously published. [7] Briefly, MSK-IMPACT is a comprehensive molecular profiling assay that involves hybridization capture and deep sequencing of all exons and selected introns of up to 468 oncogenes and tumor-suppressor genes, allowing the detection of point mutations, small and large insertions or

deletions, and rearrangements. In addition to capturing all coding regions of the genes, the assay also captures >1000 intergenic and intronic single-nucleotide polymorphisms (tiling probes), interspersed homogenously across the genome, aiding the accurate assessment of genome-wide copy number. The assay uses paired tumor / normal DNA for sequencing and analysis. In total, the probes target approximately 1.2 megabases of the human genome.

DNA Extraction and Polymerase Chain Reaction (PCR)

Genomic DNA was extracted from either frozen or formalin-fixed paraffin-embedded tissue, using the phenol/chloroform method or the QIAamp DNA FFPE Tissue Kit (Qiagen, Valencia, CA), respectively. The targeted exon regions of candidate genes (*NRAS, KRAS, HRAS*) were amplified by the PCR using corresponding forward and reverse primers (Supplementary Table 2). PCR was conducted using the Clontech Advantage 2 PCR Enzyme System kit (Clontech, Mountain View, CA). The PCR products were purified using QIAquick PCR Purification Kit (Qiagen) and confirmed by Sanger sequencing. All mutations were verified bidirectionally.

Statistical Analysis

Statistical analysis was conducted on the SPSS software platform (version 20; IBM, New York, NY). Associations between RAS mutation and clinicopathological factors were assessed by Pearson χ^2, Fisher exact or Kruskal-Wallis tests according to whether the variables were categorical or continuous. The overall survival was measured from the date of surgery and examined by Kaplan-Meier analysis and the log-rank test. Two-sided P values < 0.05 were considered significant in all statistical analyses.

RESULTS

Clinicopathologic Features

The clinicopathologic features are summarized in Table 1. Overall, 26 cases (27%) were identified with *RAS*/*RAF* mutations. These included 14 cases (54%) with *NRAS* mutation, 6 cases (23%) with *KRAS* mutation, 5 cases (19%) with *HRAS* mutation and 1 case (4%) with *BRAF* mutation (Supplementary Figure 1). The patients age at diagnosis ranged widely, from 1 to 70 years-old (median: 8 years). Two-thirds of tumors occurred in the pediatric age group, 15 patients (58%), while the remaining 11 (42%) occurred in adults. Sixteen (64%) of the patients were males and 10 (38%) were females. The most common clinical presentation was the intra-abdominal and genitourinary site, occurring in 15 (58%) patients, including 5 involving the pelvis/intra-abdominal, 6 the vagina/uterus/cervix, and 4 occurring paratesticular. The second most common location was the head and neck, occurring in 6 (23%) patients. Five patients occurred in the lower extremity (4) and axilla (1). Among the 15 pediatric tumors, 9 (60%) occurred in the intra-abdominal / genitourinary sites, 4 (27%) tumors occurred in the head and neck locations and 2 (13%) occurred in the soft tissue. Tumors ranged in size from 1.0 to 33.0 cm in greatest dimension (mean 6.3 cm). Twenty-two (85%) of the 26 patients presented with localized disease at the time of presentation.

Morphologically, all 26 tumors were classified as ERMS. Ten (10, 40%) cases showed primitive undifferentiated round to ovoid cells in compact sheets (Figure 1A-1C). In focal

areas the primitive cells were in a loose, partially myxoid, stroma (Figure 1B). Nine (9, 35%) showed admixture of undifferentiated and spindle cell areas with the spindle cell areas showing cellular spindle cells arranges in fascicles. One case (case # 25) showed a pure spindle cell morphology and one case (case #6) showed a distinctive botryoid morphology (Figure 1D). Rhabdomyoblastic differentiation with strap cells was not a common feature and was noted only in 2 cases. Mitoses ranged from 2 to 25 (mean – 11) per 10 high power fields. Seven cases (27%) showed areas of necrosis.

Immunohistochemically, all except one case showed diffuse cytoplasmic positivity for desmin. Myogenin showed focal nuclear positivity in all cases except one. MyoD1 staining was performed on 8 cases and showed focal nuclear positivity in all of the cases.

In all cases, FISH studies performed at the initial diagnosis did not show evidence of *FOXO1* gene fusions.

Clinical risk-group data was available on 24 of 26 cases in the study. Nine patients were classified in the low-risk group, 11 in the intermediate-risk group, while 4 in the high-risk group. (Table 1)

NRAS mutations in RMS predominantly occur in males with codon 61 being the most common site of mutation.

Ten of 14 (71%) *NRAS*-mutated tumors occurred in children less than 18 years of age. The remaining 4 cases occurring in patients aged 19, 34, 62 and 70 years. Nine cases occurred in males and 5 in females. *NRAS Q61K* was the most common mutation identified in 8 cases, *NRAS Q61L* and *NRAS Q61R* mutations were identified in 2 cases each, and the other 2 cases showed *NRAS Q61H* and *NRAS G13V* mutations. The tumors were located mainly in the abdomino-pelvic / genitourinary sites (7 cases), with 4 cases occurring in the soft tissues and 3 cases in the head and neck. More than half the cases showed morphology of primitive undifferentiated cells with round to ovoid nuclei in sheets. In focal areas, the tumor cells were arranged in a partially myxoid background (Figure 1A-1C, 1F). Three cases showed a combination of undifferentiated cells with areas of spindle cells (Figure 1E). One case showed a botryoid morphology (Figure 1D).

KRAS G12D is the most common *KRAS* mutation in RMS

KRAS mutations in ERMS were identified in 3 children and 3 adults, with equal gender distribution. *KRAS G12D* hot spot mutation occurred in 4 of the 6 cases. The remaining 2 cases showed *KRAS G12A* and *KRAS K117T* mutations. All tumors were located in the genitourinary sites including pelvis, paratesticular, vaginal and uterus. Three of the tumors show hybrid morphology with undifferentiated round cell areas and areas with spindle cell morphology. The other three tumors show sheets of primitive undifferentiated cells (Figure 2A, 2B).

ERMS with *HRAS* mutations have heterogeneous clinical and pathologic features.

The 5 cases with *HRAS* mutations occurred in 3 males and 2 females, affecting 1 child and 4 adults. The mutations that were identified were *HRAS G12V, HRAS G13R, HRAS Q61K,*

HRAS Q61L and *HRAS T50M*. Three tumors were located in the head and neck location with the other two located in paratesticular and thigh. Two tumors showed morphology of undifferentiated round cells, one of which had focal rhabdomyoblastic differentiation. Two other tumors showed hybrid morphology of primitive undifferentiated cells and areas with spindle cell features. One of the tumors show a pure spindle cell morphology with spindle cells arranged in fascicles. Scattered cells in this case showed large pleomorphic nuclei (Figure 2C, 2D).

One case showed a *BRAF V600E* mutation and occurred in the abdomen of a 4-year-old boy and showed primitive undifferentiated cells in a loose myxoid stroma (Figure 2E, 2F).

Additional co-existing genetic abnormalities identified.

Next-generation sequencing identified additional genetic alterations in 17 of the 20 (85%) cases studied. These are listed in Supplementary Table 1. The recurrent additional genetic alterations included 4 cases each with *TP53*, *NF1*, or *BCOR* mutations, 2 cases each with *MYC* amplification, *RAD21* amplification, *AKT3*, *PIK3CA*, *PTEN* and *FGFR4* mutations. Other non-recurrent genetic alterations identified are listed in Supplementary Table 1.

Follow-up

Clinical follow-up was available on all patients (Table 1) and ranged from 2 to 124 months (mean – 29.5 months). Fifteen patients were managed with a combination of chemotherapy, radiation therapy and surgery, 9 received surgery and chemotherapy and 2 recent cases are being treated with chemotherapy. Seven (7) patients had local recurrence, 5 patients had distant recurrence and 2 patients had local and distant recurrences. At last follow up, 13 patients (52%) had no evidence of disease (NED), 6 patients (23%) were alive with disease (AWD) and 7 patients (28%) had died of disease (DOD) at 2 to 64 months following diagnosis. The 3-year and 5-year survival rates for the entire cohort were 75% and 61% respectively, while for patients presenting with localized disease, the 3-year and 5-year survival rates were 81% and 65% respectively. No significant differences in survival were noted among patients with different *RAS* mutations. (Supplementary Figure 2)

Among the *NRAS* mutated group, 3 of the patients died of disease at 3, 15 and 34 months following diagnosis; 3 were alive with disease and 8 had no evidence of disease. Among the *KRAS* mutated group, 2 patients died of disease at 4 and 12 months following diagnosis, 1 was alive with disease at 28 months and 3 had no evidence of disease. Among the *HRAS* mutated group, 2 patients died of disease at 54 and 64 months following diagnosis, 1 was alive with disease at 5 months and 2 had no evidence of disease. The *BRAF* mutated patient was alive with disease at 8 months following diagnosis.

DISCUSSION

RAS-ERK (RAS-RAF-MEK-ERK) pathway is a signal transduction cascade affecting cell proliferation and survival. [8, 9] Various growth factors bind to their receptors and activate RAS which, in turn, activates RAF and results in MEK phosphorylation. Phosphorylated MEK then phosphorylates ERK which activates various cell cycle-related proteins. This pathway has been implicated in the oncogenesis of various hematological and solid tumors.

Activating RAS mutations occur in approximately 30% of human cancers. [10, 11] The RAS family, including HRAS, KRAS, and NRAS, belongs to the small G protein superfamily and involves the Ras/MAPK (mitogen-activated protein kinase) pathway, which transduces the extracellular input to the intracellular compartment through the GDP/GTP-regulated switches. The cancer-related somatic mutations in RAS members often occur at amino acids 12, 13, or 61, which are the conserved sites for modulating GDP/GTP binding and hydrolysis. There is increasing evidence that Ras proteins have isoform-specfic biological functions and tumorigenic effects, possibly attributed to the C-terminal hypervariable region, and that different mutation codons might induce different transformation potential.[11] Different tumor types often display specificity in *RAS* gene involvement. For example, *HRAS* mutations are prevalent in bladder and kidney carcinoma, while *KRAS* mutations predominate in colorectal, pancreatic, endometrial, lung, and cervical cancers, and *NRAS* lesions are more commonly observed in melanoma and liver carcinoma as well as in lymphoid and myeloid malignancies.[12]

The RAS/MEK/ERK pathway also plays a major role in RMS [13] as it can trigger uncontrollable proliferation of cancer cells and prolong their survival time. [14-16] Mutations in RAS in zebrafish have been shown to lead to development of ERMS. [17, 18] Previous studies have shown that *RAS* gene mutations can be identified in 5-30% of all fusion negative RMS patients. [2, 12, 19-22] Shern et al. observed some age-specific correlations in *RAS* mutated RMS patients with *HRAS* mutations occurring in infants, *KRAS* mutations in toddlers and *NRAS* mutations in adolescents.[4]

In our study, *RAS / RAF* mutations were seen in 27% of 98 ERMS studied. Similar to prior studies [2-4, 12, 22], *NRAS* gene mutations were the most prevalent, accounting for half of the cases. *KRAS* and *HRAS* gene mutations occurred at a similar rate, comprising a quarter of RAS mutant ERMS. *BRAF* mutation was identified in only one case, indicating that *RAS* gene mutations far outnumber the *RAF* gene mutations in RMS. Mutations in all of the genes were missense mutations and occurred predominantly in the well-known hotspots described in various cancers. Codon 61 was the most common site of *NRAS* mutations, seen in 13 of the 14 *NRAS*-mutated tumors. One tumor showed a codon 13 mutation, another hotspot for *NRAS* gene. All except one of the *KRAS* mutations were seen in codon 12. Only one case showed codon 117 mutation, a rare site of *KRAS* mutations. Similarly, all except one *HRAS* mutations were identified in hot spot regions of codons' 61, 12 and 13. One case showed instead an infrequent site mutation in codon 50 mutation. The *BRAF* mutated RMS occurred in the hot spot codon 600. The significance of the rare non-hotspot *RAS* mutations such as *KRAS p.K117T* and *HRAS p. T50M,* with respect to being 'driver' or 'passenger' mutations, remains uncertain.

Most of the RAS / RAF mutated tumors in our study analyzed by next-generation sequencing platform showed additional genetic alterations. *TP53* gene alterations were the most common additional genetic alterations seen in 4 cases. Interestingly, a number of co-mutated genes such as *NF1, FGFR4, PIK3CA, AKT3*, etc, are part of the *RAS / RAF / AKT* pathway, which raises an important question as to the significance of these different mutations in the pathogenesis of ERMS. These results are in keeping with those reported by

other groups.[2-4] Three cases also showed evidence of coexistent loss of function mutations in *BCOR* gene, which has been previously described. [3]

HRAS mutations have also been reported in malignant ectomesenchymoma (MEM), a rare multi-phenotypic sarcoma that occurs in pediatric age group and composed of mesenchymal component in the form of rhabdomyosarcoma and an intimately admixed neuroectodermal component in the form of ganglioneuroblastoma or ganglioneuroma. In a recent study by Huang et al. [23] 6 of the 7 malignant ectomesenchymoma cases showed *HRAS* mutations, 4 showing p.G13R mutation and 2 showing p.Q61L mutation. Most of the tumors had an ERMS like morphology. In unsupervised hierarchical clustering based on gene expression data, the MEM cases grouped tightly with the RMS cases. We have since seen 3 additional cases of MEM, all with *HRAS* mutations (2 with G13R and 1 with Q61L) and, similar to the prior study, were mostly located in the GU area. In the study from Huang et al.[23], none of the cases with available follow-up had died of disease. The 3 additional cases we have seen also had no evidence of disease at last follow-up. This suggests that, with similar therapy, MEM have a better prognosis than *RAS*-mutated ERMS. The shared clinicopathologic, molecular and gene expression findings suggest that MEM has a close relationship to ERMS.

The impact of *RAS / RAF* mutations on prognosis of ERMS is uncertain, based on current literature. Although some studies suggest that *RAS* mutations are significantly associated with intermediate- and high-risk ERMS [24], other studies have shown that *RAS* gene mutations do not portend a poor prognosis.[4] In our study, half (48%) of the *RAS / RAF* mutated RMS patients were either alive with disease or had died of the disease in spite of multimodal management including surgery, chemotherapy and radiation therapy. For patients presenting with localized disease, the 3-year and 5-year survival rates were 81% and 65% respectively. Although comparable, this is marginally lower than the reported 70% 5-year survival of ERMS patients presenting with localized disease. The presence and type of additional genetic alterations, as identified by next-generation sequencing molecular studies, did not seem to have a consistent impact on either survival or response to multimodal management including chemotherapy, radiation therapy and surgery.

The significance of RAS mutations from a therapeutic aspect is not certain. Most of our patients were treated with standard rhabdomyosarcoma regimens. A recent study [25] showed that a novel RAF/MEK inhibitor CH5126766 causes G1 cell cycle arrest in *RAS*-mutated RMS cell lines. More recently, inhibitors specifically targeting the *KRAS p. G12C* (AMG-510, MRTX-849, and JNJ-74699157)[26] have been developed with AMG-510 obtaining FDA approval for treatment of adult patients with *KRAS p. G12C* mutated locally advanced or metastatic non-small cell lung cancer. This holds great promise in treatment of other tumors with *KRAS p. G12C* mutations. Additional studies are needed to study the efficacy of these targeted drugs in patients with *RAS*-mutated RMS.

In conclusion, *RAS* mutations are noted in approximately a quarter of all ERMS. *NRAS* gene mutations constitute one-half of these mutations, while *KRAS* and *HRAS* gene mutations account for the remaining cases. Most of the *RAS*-mutant RMS showed additional genetic alterations including mutations in genes affecting the RAS/RAF/ERK pathway.

Although *RAS* mutated ERMS in our study appeared to have similar survival rates to that reported in all ERMS, additional larger studies are necessary to determine the true prognostic impact of *RAS*/*RAF* mutations. Although there was no statistically significant correlation, most *RAS*-mutant ERMS occurred in children and in the H&N and GU anatomic locations. In fact, all KRAS mutant ERMS were located in the GU area. All except two ERMS characterized by a *RAS*-positive genotype harbored an undifferentiated and/or spindle cell morphology lacking the typical strap or rhabdomyoblastic type cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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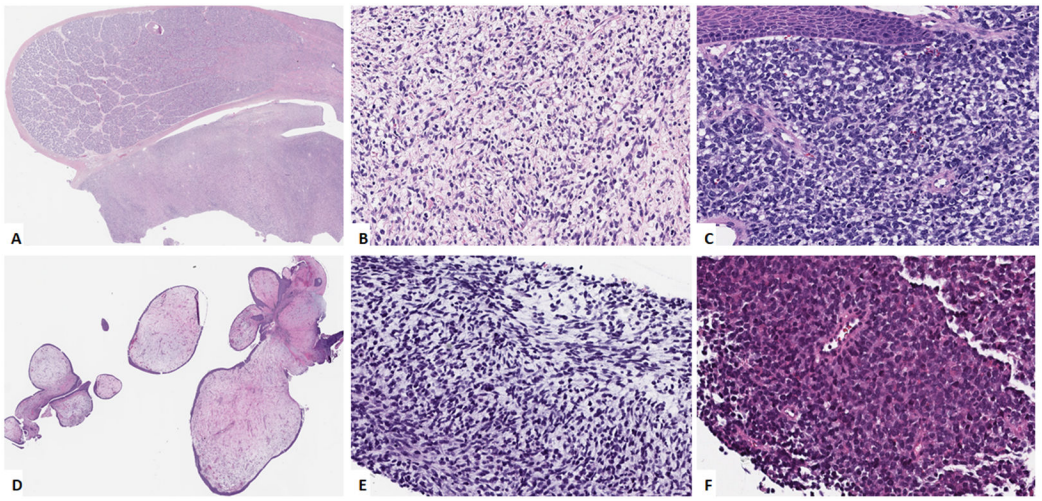

Figure 1: Morphologic spectrum of *NRAS*-mutated RMS.

A, B. (case 2, 2/M) Paratesticular tumor (A) showing primitive appearing cells in a loose stroma (B). C. Soft palate lesion (case 3, 2/M) composed of undifferentiated cells with round to irregular nuclei arranged in sheets, involving submucosa. D. Vaginal tumor (case 5, 3/F) showing botryoid morphology with polypoid nodulear growth. E. Jaw lesion (case 6, 5/M) showing primitive ovoid to spindle cells in a myxoid background. F. Calf tumor (case 11, 62/M) showing undifferentiated small cells arranged in sheets.

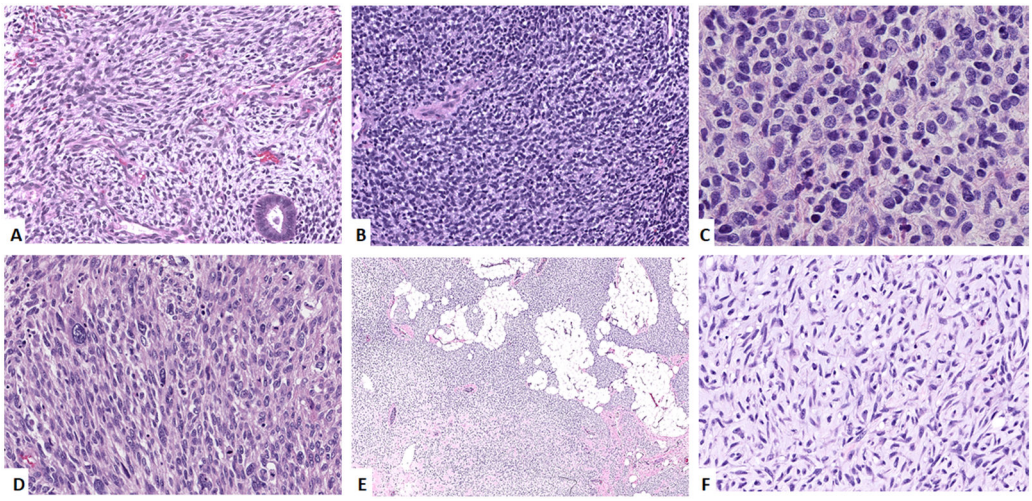

Figure 2: Morphologic spectrum of *KRAS*- (A-C), *HRAS*- (D) and *BRAF*-mutated (E, F) ERMS.
A. Uterine tumor (case 19, 44/F) showing primitive-appearing spindle cells involving the endometrium. B. Vaginal tumor (case 16, 1/F) showing undifferentiated cells with round nuclei in sheets. C. Paratesticular mass (case 22, 20/M) showing sheets of undifferentiated round cells. D. Thigh lesion (case 25, 50/M) showing a sarcomatoid morphology with spindle to pleomorphic cells in a fascicular pattern. E, F Abdominal tumor (case 26, 4/M) showing a cellular tumor infiltrating adipose tissue. Higher power image (F) showing primitive spindle cells in a collagenous to myxoid stroma.

Table 1:

Clinico-pathological features of *RAS / RAF* mutated RMS

Case #	Age (yrs)	Sex	Site	RAS / RAF mutation	Risk Group	FU duration (months)	FU status	Treatment
1[¥]	1	F	buttock	*NRAS exon3 p.Q61K (c.181 C>A)*	High	15	DOD	S+C
2	2	M	Para-testicular	*NRAS exon3 p.Q61K (c.181C>A)*	Low	48	NED	S+C
3[¤]	2	M	soft palate	*NRAS exon3 p.Q61K (c.181C>A)*	Low	15	AWD	S+C
4[§]	2	F	thigh	*NRAS exon3 p.Q61K (c.181C>A)*	NA	19	NED	C+R+S
5[¤]	3	M	pelvis	*NRAS exon3 p.Q61H (c.183A>T)*	Int	16	NED	C+R+S
6	3	F	vagina	*NRAS exon3 p.Q61K (c.181C>A)*	Low	17	NED	C+R+S
7	5	M	jaw	*NRAS exon2 p.G13V (c.38G>T)*	Int	22	NED	S+C
8	7	M	Para-testicular	*NRAS exon3 p.Q61K (c.181C>A)*	Low	36	NED	S+C
9[¤]	9	M	infratemporal fossa	*NRAS exon3 p.Q61L (c.182A>T)*	Int	55	NED	C+R+S
10	10	M	abdomen	*NRAS exon3 p.Q61R (c.182A>G)*	Int	3	DOD	C+S
11[§]	19	F	vagina	*NRAS exon3 p.Q61K (c.181C>A)*	Int	34	DOD	C+R+S
12	34	F	cervix	*NRAS exon3 p.Q61R (c.182A>G)*	Int	2	AWD	C
13[¤]	62	M	calf	*NRAS exon3 p.Q61K (c.181C>A)*	Int	60	NED	C+R+S
14	70	M	axilla	*NRAS exon3 p.Q61L (c.182A>T)*	Int	35	NED	C+R+S
15	1	M	pelvis	*KRAS exon 2 p.G12D (c.35G>A)*	High	12	DOD	C+R+S
16[¤]	1	F	vagina	*KRAS exon2 p.G12D (c.35G>A)*	Low	28	AWD	C+R+S
17	2	M	pelvis	*KRAS exon 2 p.G12D (c.35G>A)*	Int	31	NED	C+R+S
18[¥]	23	M	Para-testicular	*KRAS exon4 p.K117T (c.350A>C)*	High	52	NED	C+R+S
19	44	F	uterus	*KRAS exon2 p.G12A (c.35G>C)*	Low	19	NED	S+C
20[¥]	62	F	endometrium	*KRAS exon2 p.G12D (c.35G>A)*	NA	4	DOD	S+C
21[¤]	3	F	orbit	*HRAS exon3 p.Q61K (c.181C>A)*	Low	39	NED	C+R+S
22[¥]	20	M	Para-testicular	*HRAS exon2 p.G12V (c.35G>T)*	Low	54	DOD	C+R+S
23	22	F	buccal	*HRAS exon 3 p.Q61L*	Low	124	NED	C+R+S
24[¤]	32	M	pterygoid fossa	*HRAS exon 2 p.G13R*	Int	64	DOD	C+R+S
25	50	M	thigh	*HRAS exon3 p.T50M (c.149C>T)*	Int	5	AWD	S+C
26[¥]	4	M	abdomen	*BRAF exon15 p.V600E (c.1799T>A)*	High	8	AWD	C

F-female, M-male, int-intermediate, NA-not available, DOD-died of disease, AWD-alive with disease, NED-no evidence of disease, C-chemotherapy, S-surgery, R-radiation therapy

[¥]-Distant recurrence

[¤]-Local recurrence

[§]-Local and distant recurrence